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Metabolism of apolipoprotein E-containing human plasma lipoproteins by rat and human cells in culture

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Cultured preadipocytes from rat epididymal fat pads were able to bind, internalize, and degrade human plasma very-low-density lipoproteins (VLDL) more efficiently than low-density lipoproteins (LDL). VLDL, but not LDL, activated acyl-CoA:cholesterol acyltransferase (ACAT) and increased cholesterol accumulation in these cells. However, trypsin-treated VLDL (T-VLDL) lost the capacity to bind, activate ACAT, and increase cholesterol accumulation. After the treatment of VLDL with trypsin, SDS/polyacrylamide-gel electrophoresis and immunoblotting showed that apolipoprotein E (apo E) was completely degraded, whereas apolipoprotein CII (apo C-II) was preserved. ApoE complexed with dimyristoyl phosphatidylcholine (DMPC) was able to complete with VLDL for binding to the cells. Although T-VLDL did not bind to the preadipocytes, these cells accumulate triacylglycerols from T-VLDL, presumably after lipolysis, as efficiently as from native VLDL. Rat smooth muscle cells and skin fibroblasts also bind and metabolize human VLDL better than LDL. However, human skin fibroblasts and omental preadipocytes metabolized LDL better than VLDL. These studies indicate that rat tissues can recognize and metabolize apoE-containing human plasma VLDL although they cannot recognize human LDL.

INTRODUCTION

Adipose tissue has been recognized as one of the largest triacylglycerol and cholesterol storage organs in the body (Angel *et al.*, 1979; Farkas *et al.*, 1973). Although extensive studies have been carried out on the metabolism of triacylglycerols in this tissue, very little work was directed toward the understanding of cholesterol metabolism (Krause & Hartman, 1984). The importance of LDL in the metabolism of cholesterol has been studied in detail by Goldstein and Brown (Goldstein *et al.*, 1976). However, the significance of LDL in the metabolism of cholesterol in adipose tissue is not clearly understood. It has been reported that LDL can be bound and degraded by human adipose cells, and high density lipoproteins (HDL), VLDL, and modified LDL can compete with these processes (Angel & Fong, 1983; Fong *et al.*, 1984). They suggested that there is a relaxed specificity of the fat cell for various lipoproteins (Fong *et al.*, 1984). Desai *et al.* (1980) reported that there are specific saturable receptors for VLDL on isolated rat and human adipocytes, and apo CII was proposed as ligand for the binding.

It has been shown that rat skin fibroblasts metabolize rat plasma LDL much faster than human plasma LDL (Drevon *et al.*, 1981; Innerarity *et al.*, 1980). In the present studies we investigated the metabolism of human plasma VLDL and LDL in rat epididymal preadipocytes, mesenteric arterial smooth muscle cells and skin fibroblasts and human omental preadipocytes and skin fibroblasts in culture. The results suggests that the rat cells have receptors which can recognize human apo E but not human apo B.

EXPERIMENTAL

Materials

$Na^{125}I$ (carrier-free) was obtained from Amersham Corp. [^{14}C]Oleic acid, [^{3}H]cholesteryl oleate and [^{14}C]triolein were obtained from New England Nuclear. Bovine serum albumin (essentially fatty acid free), elastase, trypsin, trypsin inhibitor, cholesteryl oleate, sodium oleate, triolein and DMPC were purchased from Sigma. Collagenase was obtained from Worthington. Peroxidase-conjugated anti-(rabbit IgG) was obtained from Miles. β-Sitosterol was obtained from Applied Science (State College, PA, U.S.A.). All tissue culture supplies were purchased from Grand Island Biological.

Isolation of VLDL and LDL

Normal human plasma was obtained from a blood bank, and NaN_3 (0.01%), EDTA (1 mM), and gentamycin (0.1 mg/ml) were added. VLDL and LDL were isolated by sequential ultracentrifugation as described previously (Yamamoto *et al.*, 1985). The LDL preparations had 2 mg and 0.5 mg of cholesterol and triacylglycerol respectively per mg of protein. The corresponding values for VLDL were 1.1 and 5 mg/mg of protein. LPDS was prepared by the method described by Radding & Steinberg (1960). Iodination of lipoproteins with $Na^{125}I$ and ICl was carried out as described earlier (Bilheimer *et al.*, 1972). T-VLDL was prepared as described previously (Yamamoto *et al.*, 1985).

Isolation and culture of preadipocytes

Epididymal fat pads from adult Sprague–Dawley rats (250–350 g) or human omental fat removed during

Abbreviations used: LDL, low-density lipoproteins: VLDL, very-low-density lipoproteins; T-VLDL, trypsin-treated VLDL; apo E, B and C, apolipoproteins E, B and C; DMEM, Dulbecco's modified Eagle's minimum essential medium; LPDS, lipoprotein-deficient serum; DMPC, dimyristoyl phosphatidylcholine; ACAT, acyl-CoA:cholesterol acyltransferase; LPL, lipoprotein lipase.

surgery were washed, minced, and incubated with collagenase (1.5 mg/ml in phosphate-buffered saline) for 20–30 min at 37 °C with gentle stirring. The digest was passed through 250 μm nylon mesh and centrifuged at 1200 *g* for 10 min. The supernatant was discarded, and the pellet containing the stromal cells was suspended in DMEM containing penicillin (100 units/ml), streptomycin (100 μg/ml), Hepes (25 mM, pH 7.4), and fetal calf serum (10%) in 75 cm^2 tissue culture flasks. The flasks were incubated in a humidified incubator with under air/CO_2 (19:1) at 37 °C. The medium was changed on alternate days. After the monolayers were confluent, the cells were subcultured using trypsin (37 °C, 3–4 min) into 60 mm tissue culture dishes at a concentration of 1×10^5 cells/dish. The cells were used after 6–8 days when they became confluent.

Rat mesenteric arterial smooth muscle cells and skin fibroblasts

Vascular smooth muscle cells were isolated enzymically from rat mesenteric artery according to the method of Gunther *et al.* (1982). Rat skin fibroblasts were grown from explants of skin from the abdominal wall.

Binding and degradation of lipoproteins in preadipocytes

The binding and degradation of ^{125}I-labelled LDL and VLDL were studied according to the methods described previously (Brown & Goldstein, 1974; Goldstein & Brown, 1983). Briefly, after 6–8 days of growth, when the monolayers were confluent, the cells were washed and incubated in DMEM containing 5% LPDS for 48 h. At the end of this period, the medium was replaced with fresh DMEM containing 5% LPDS. The binding of ^{125}I-labelled LDL and VLDL was studied at 4 °C for 3 h. After incubation, the monolayers were washed three times using phosphate-buffered saline containing bovine serum albumin and three times using the same buffer without albumin and then dissolved in 0.2 M-NaOH, and the radioactivity was determined. To study the degradation of the lipoproteins, the cells were incubated with ^{125}I-labelled LDL and VLDL at 37 °C for 5 h. The medium was collected, and the trichloroacetic acid-soluble non-iodide radioactivity was determined as described elsewhere (Goldstein *et al.*, 1977). In the same experiment, the cell-associated radioactivity was determined after extensive washing of the monolayers. All the lipoprotein additions and amounts metabolized by the cells are expressed in terms of protein.

Acyl-CoA:cholesterol acyltransferase

After incubating monolayers for 48 h in DMEM containing 5% LPDS, they were incubated with 0.1 mM-[1-^{14}C]oleate bound to bovine serum albumin and the respective lipoproteins for 20 h at 37 °C, and the radioactivity incorporated in cholesteryl oleate was determined as described earlier (Goldstein *et al.*, 1975).

Lipoprotein lipase

To determine the lipoprotein lipase activity, the confluent monolayers were incubated with medium containing 10% fetal calf serum and heparin (10 units/ml) for 16 h. The medium was collected, and the release of [^{14}C]oleic acid from [^{14}C]triolein emulsion was determined as described by Nilsson-Ehle & Schotz (1976).

Isolation of apo E and preparation of DMPC–apo E vesicles

Apo E was isolated from human VLDL by the method described by Weisgraber *et al.* (1977). DMPC vesicles were prepared by sonicating 10 mg of DMPC in 1 ml of phosphate-buffered saline for 10 min in an ice/water bath. Apo E–DMPC complexes were prepared by incubating DMPC vesicles and apo E as described previously (Innerarity *et al.*, 1979).

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out in 3–20% gradient or 12.5% slab gels containing 0.25 M-Tris/HCl, pH 9, and 0.1% SDS. The electrophoresis buffer was made with 25 mM-Tris, 0.2 M-glycine and 0.1% SDS, pH 8.4. After electrophoresis at 20 mA, the gel was stained with Coomassie Blue R.

Immunoblotting

After SDS gel electrophoresis of lipoproteins, the proteins were transferred from the gel onto a nitrocellulose sheet according to the method of Towbin *et al.* (1979). The nitrocellulose sheets were incubated for 1 h at 37 °C in a buffer containing 20 mM Tris/HCl, pH 7.4, 0.15 M-NaCl, and 1% bovine serum albumin. After this incubation, antibodies to apo E or apo C-II raised in rabbits were added, and the incubation was continued for 2 h at room temperature. The sheets were then washed with the same buffer without bovine serum albumin for 30 min with three changes and incubated with anti-(rabbit IgG) conjugated with peroxidase for

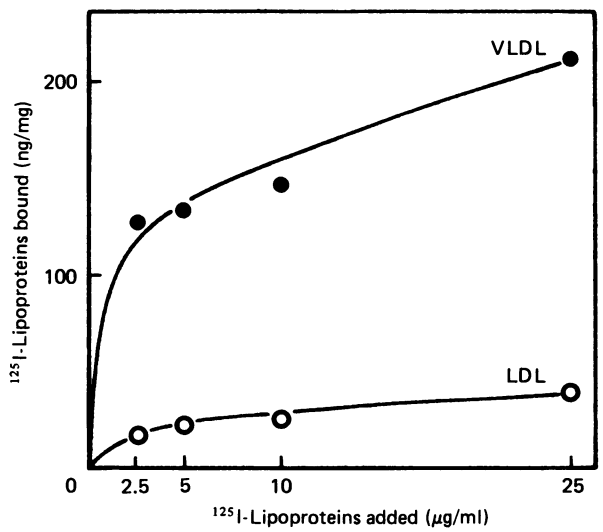

Fig. 1. Specific binding of ^{125}I-labelled VLDL and LDL to rat epididymal preadipocytes

After the preadipocytes had been incubated with medium containing LPDS for 48 h, the medium was removed and replaced with fresh medium. The cells were cooled to 4 °C and incubated with the indicated amounts of ^{125}I-labelled VLDL (170 c.p.m./ng) or LDL (125 c.p.m./ng) for 3 h. The bound VLDL or LDL was determined as described in the Experimental section. Nonspecific binding was determined by the addition of a 10-fold excess of unlabelled VLDL or LDL, respectively. Specific binding was obtained by subtracting the nonspecific binding from the total binding.

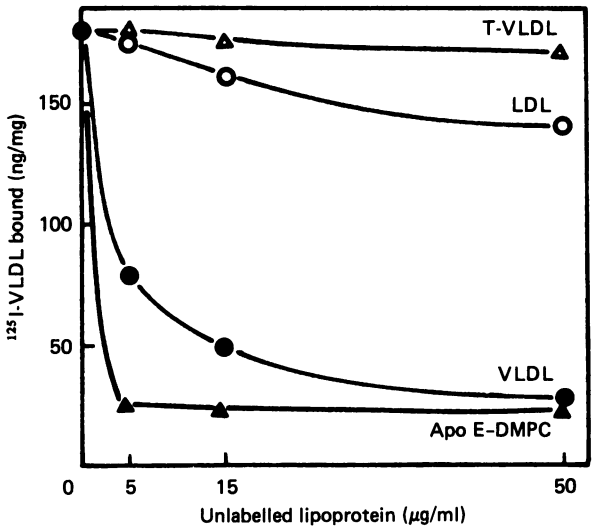

Fig. 2. Effect of unlabelled lipoproteins on binding of ^{125}I-VLDL to preadipocytes

After the preadipocytes had been incubated with medium containing LPDS for 48 h, the medium was removed, and 2 ml of fresh medium containing LPDS was added. The cells were cooled to 4 °C and incubated with ^{125}I-VLDL (5 μg/ml, 192 c.p.m./ng) and indicated concentrations of unlabelled lipoproteins for 3 h at 4 °C. The cell-associated ^{125}I-VLDL was determined as described in the Experimental section.

2 h. After washing, the antigens were visualized using H_2O_2 and 4-chloro-1-naphthol (Bio-Rad).

Determination of cellular cholesterol and triacylglycerols

The monolayers were incubated with medium containing 5% LPDS with or without lipopoproteins for 24 h at 37 °C. At the end of this period, the cells were washed, and the lipids were extracted with hexane/propan-2-ol (3:2, v/v). Total cholesterol was determined after saponification by g.l.c. using a glass column packed with 3% OV-17 at 270 °C. β-Sitosterol was used as internal standard. Triacylglycerols were determined by using the kit obtained from Beckman.

Presentation of results

Values presented in the Figures and Tables are means of triplicate determinations.

RESULTS

The binding capacity of ^{125}I-labelled human LDL and VLDL at 4 °C was studied in preadipocytes derived from rat epidiymal fat pads. As shown in Fig. 1, the specific binding of VLDL to the preadipocytes increased with the increasing concentration of VLDL. This binding was saturable reaching a maximum at a concentration as low as 5 μg/ml, indicating the presence of high-affinity receptors on the cell surface. However, the binding of LDL to these cells was very low. To investigate the nature of the receptors, we studied the displacement of ^{125}I-labelled VLDL by unlabelled lipoproteins. The results shown in Fig. 2 indicate that VLDL compete with the labelled VLDL for binding to the specific receptors very efficiently, whereas LDL had very little effect. Trypsin treatment of VLDL resulted in the complete degradation of apo E from the particle (Fig. 3). The T-VLDL was not able to compete with the labelled VLDL, as shown in Fig. 2, suggesting that apo E is involved in the binding of VLDL to the receptors. To confirm this possibility, the displacement of labelled VLDL with apo E–DMPC complex was studied. The results shown in Fig. 2 indicate that apo E–DMPC complex can compete with labelled VLDL for the binding very effectively. These results strongly suggest that rat preadipocytes possess specific receptors for apo E and very little or no specific receptors for apo B from human lipoproteins. Desai *et al.* (1980) have reported that VLDL bind to human and rat adipocytes, and they concluded that apo C-II is the ligand for the VLDL binding. However, this does not appear to be true in our experiments. Our results strongly suggest that preadipocytes possess specific receptors for apo E and very little or no specific receptors for human apo B. Apo E was completely degraded into smaller fragments in T-VLDL and these fragments reacted with antibodies against apo E as detected by immunoblotting (Fig. 4). However apo C was intact in T-VLDL as determined by SDS/polyacrylamide gel electrophoresis (Fig. 3). Immunoblotting studies further confirmed that apo C-II is not significantly altered by trypsin treatment (Fig. 4).

To investigate if rat adipocytes can take up cholesterol from human VLDL, activation of ACAT and accumula-

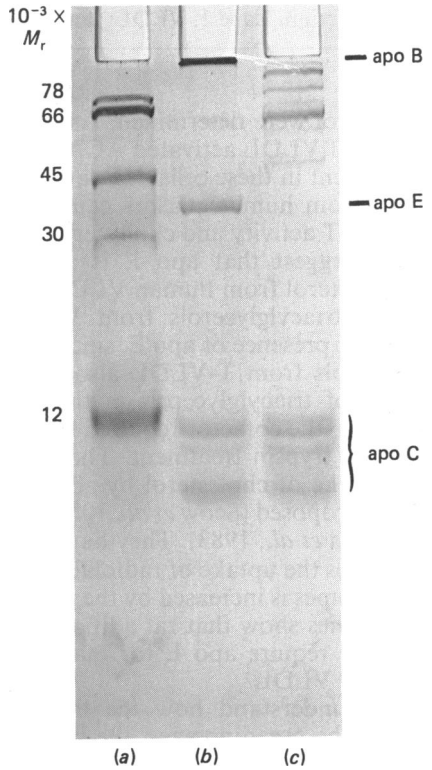

Fig. 3. SDS/polyacrylamide-gel electrophoresis of VLDL and T-VLDL

VLDL were incubated with trypsin at the protein ratio of 100:1 for 16 h at 37 °C and reisolated by centrifugation. Approx. 25 μg of protein was subjected to electrophoresis in a 12.5% polyacrylamide gel containing 0.1% SDS. Lane *a*, molecular weight standards; lane *b*, VLDL; lane *c*, T-VLDL.

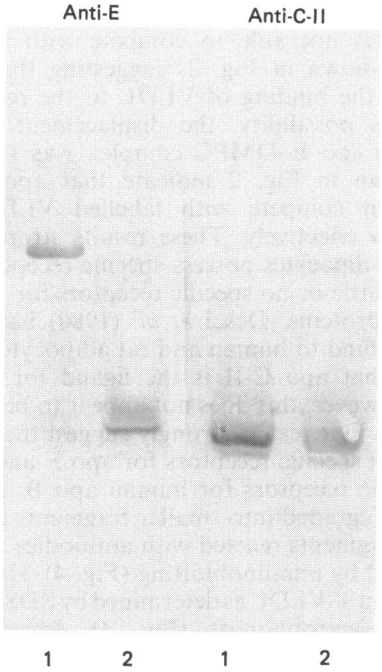

Fig. 4. Immunoblotting of VLDL and T-VLDL

After electrophoresis of 25 μg of each sample in 3–20% gradient polyacrylamide gel containing 0.1% SDS, the proteins were transferred to nitrocellulose sheets electrophoretically. They were incubated with antibodies against apo E or apo C-II and processed as described in the Experimental section. Lane 1, VLDL; lane 2, T-VLDL.

tion of cholesterol were determined. As shown in Table 1, the addition of VLDL activated ACAT and increased cholesterol content in these cells. However, neither LDL nor T-VLDL from human plasma caused a significant increase in ACAT activity and cholesterol accumulation. These results suggest that apo E is required for the uptake of cholesterol from human VLDL. Nevertheless, the uptake of triacylglycerols from VLDL does not depend upon the presence of apo E, since these cells take up triacylglycerols from T-VLDL also, presumably by the hydrolysis of triacylglycerols in these particles by LPL as apo C-II, the cofactor required for LPL activity, is unaffected by trypsin treatment. The involvement of LPL in the uptake of cholesterol by adipose tissue and heart has been proposed (Scow *et al.*, 1977; Chajek-Shaul *et al.*, 1982; Stein *et al.*, 1983). They have suggested that in many cell types the uptake of radiolabelled cholesteryl ester from liposomes is increased by the presence of LPL. The present studies show that rat adipocytes, in spite of possessing LPL, require apo E for maximal uptake of cholesterol from VLDL.

In order to understand how the lipoproteins were metabolized in the preadipocytes, the binding, internalization, and degradation of ^{125}I-labelled human LDL and VLDL were studied. The results shown in Table 2 indicate that the rat preadipocytes bind, internalize, and degrade human VLDL nearly three times more than human LDL. To investigate the involvement of lysosomal degradation in the metabolism of lipoproteins in these cells, the effect of chloroquine, which is known to inhibit the lysosomal enzymes by increasing the intralysosomal pH, was studied (Goldstein *et al.*, 1978). When

Table 1. Effect of LDL, VLDL, and T-VLDL on ACAT activity, and accumulation of cholesterol and triacylglycerols in cultured rat preadipocytes

The cells were incubated with medium containing LPDS for 48 h. At the end of this incubation, each plate received 2 ml of fresh medium and 50 μg of lipoproteins/ml were added. To determine ACAT activity, 0.1 mM-[1-^{14}C]oleate was added, and the dishes were incubated for 20 h. After washing the cells, total lipids were extracted, and cholesteryl ester was separated by t.l.c. To determine total cholesterol and triacylglycerols the cells were incubated with lipoproteins for 24 h, and total lipids were extracted after washing the cells. Total cholesterol was determined by g.l.c. after saponification and the values presented are means ± S.D. of five independent determinations. Triacylglycerols were determined enzymically with a Beckman kit. The results are expressed as nmol or μg/mg of cell protein. Abbreviation: n.d., not determined.

Additions (50 μg/ml)	ACAT (nmol/ 20 h per mg)	Total cholesterol (μg/mg)	Triacylglycerols (μg/mg)
None	1.6	37.2 ± 2.5	61.2
LDL	2.2	42.4 ± 5.1	69.2
VLDL	16.3	59.4 ± 5.1	178.4
T-VLDL	1.5	36.3 ± 5.7	230.4
VLDL + chloroquine (25 μM)	1.5	n.d.	n.d.

Table 2. Binding, internalization, and degradation of ^{125}I-labelled VLDL and LDL in rat preadipocytes

After the preadipocytes were incubated with medium containing LPDS for 48 h, the medium was removed and replaced with fresh medium. The binding was determined at 4 °C for 3 h. The cell association and degradation were determined at 37 °C for 5 h. ^{125}I-labelled lipoproteins were added at 10 μg/ml. The details of the experiments are described in the Experimental section.

	Lipoprotein (ng/mg of cell protein)		
	Bound	Cell-associated	Degraded
VLDL	110	313	1166
LDL	38	115	367

chloroquine was added to the medium during the incubation, the degradation of both LDL and VLDL was inhibited (results not shown). Chloroquine also prevented the activation of ACAT by VLDL (Table 1). These findings indicate that VLDL are degraded in the lysosomes similar to LDL degradation in human skin fibroblasts (Goldstein *et al.*, 1978).

The experiments with preadipocytes from human fat tissue showed that these cells bind and metabolize LDL more efficiently than VLDL. Data shown in Fig. 5 indicate that these cells bind LDL about three times more than VLDL. Activation of ACAT activity is also more with LDL than with VLDL (Table 3). Similar results were obtained with human skin fibroblasts. To investigate if the selective binding of VLDL is characteristic of all rat

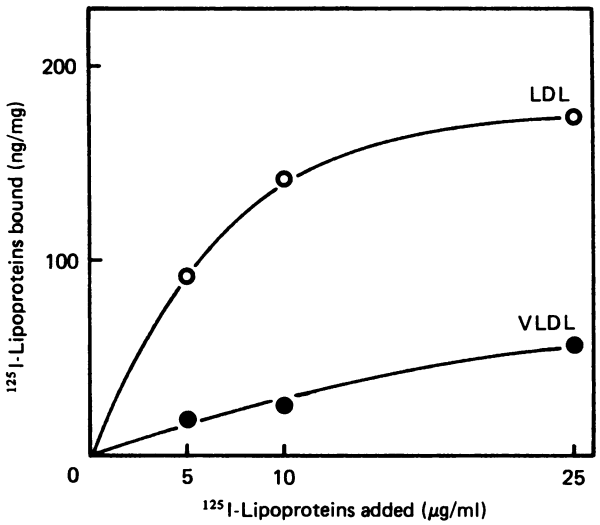

Fig. 5. Specific binding of ^{125}I-labelled VLDL and LDL to human omental preadipocytes

Experimental details are as decribed in the legend for Fig. 1.

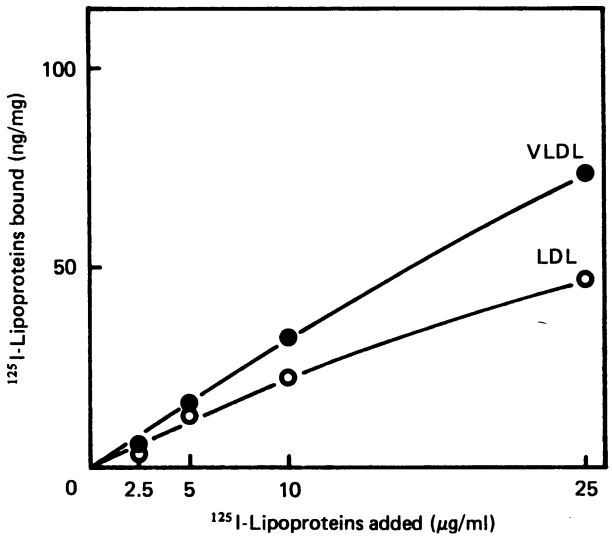

Fig. 6. Specific binding of ^{125}I-labelled VLDL and LDL to smooth muscle cells from the rat mesenteric artery

The experimental details are as described in the legend for Fig. 1.

Table 3. Stimulation of ACAT activity by LDL and VLDL in human skin fibroblasts and omental preadipocytes

The cells were incubated with medium containing LPDS for 24 h. At the end of incubation, each plate received the indicated amounts of lipoproteins. ACAT activity was determined as described by Goldstein *et al.* (1975).

Additions (50 μg/ml)	[^{14}C]Cholesteryl oleate formed (nmol/20 h per mg of protein)	
	Skin fibroblasts	Omental preadipocytes
None	0.3	0.6
LDL	25.0	52.9
VLDL	14.0	25.0

Table 4. Binding of ^{125}I-labelled LDL and VLDL to human skin fibroblasts, human preadipocytes, rat skin fibroblasts and rat preadipocytes

After the cells were incubated with medium containing LPDS for 48 h, the binding was determined as described in the Experimental section.

	^{125}I-Lipoproteins bound (ng/mg of protein)			
	Human skin fibroblasts	Human omental preadipocytes	Rat skin fibroblasts	Rat epididymal preadipocytes
LDL	118.0	77.0	9.3	4.9
VLDL	31.4	25.6	47.2	58.4

cells, the binding of VLDL and LDL was studied in rat skin fibroblasts and mesenteric arterial smooth muscle cells. These cells were also able to bind VLDL better than LDL (Table 4 and Fig. 6). The stimulation of ACAT activity by VLDL was two to three times higher than that obtained by LDL in rat skin fibroblast and smooth muscle cells (results not shown).

DISCUSSION

Although adipose tissue contains nearly 25% of the body cholesterol in the normal human and about 70% in massively obese subjects, very little is known about the metabolism of cholesterol in this tissue (Angel *et al.*, 1979; Krause & Hartman, 1984). Desai *et al.* (1980) have reported that VLDL bind to human and rat adipocytes, and they concluded that apo C-II was the ligand for the binding. This conclusion was based upon the displacement of bound VLDL by a disproportionately large excess of purified apo C-II. In our experiment, apo E–DMPC complex was 10 times more efficient compared with VLDL in displacing cell-bound ^{125}I-labelled VLDL. Apo E constitutes about 10% of the total VLDL protein. The present studies also showed that T-VLDL, which had lost apo E but not apo C-II, did not bind to the cells. These studies indicate that apo E is the ligand involved in the recognition of VLDL by the rat preadipocytes, and that apo B and apo C-II have very little role in this process. Recently, Bradley *et al.* (1984) and Bradley & Gianturco (1986) have reported that VLDL from hypertriacylglycerolaemic subjects bind to human skin fibroblasts through apo E. We observed that VLDL from normal subjects also bind to human skin fibroblasts through apo E, but not apo B (Yamamoto *et al.*, 1985).

It has been previously reported that human adipocytes can bind, internalize, and degrade LDL (Angel & Fong, 1983; Fong *et al.*, 1984). However, they neither compared LDL binding with VLDL binding nor did they study the significance of LDL in the metabolism of cholesterol. Our experiments with preadipocytes from

human fat tissue indicate that these cells bind both LDL and VLDL. The difference in the binding capacity and the activation of ACAT activity between LDL and VLDL varied from experiment to experiment, but LDL was more efficient than VLDL in all the experiments,

The results presented in this paper show that rat cells are able to recognize apo E-containing human plasma lipoproteins although they do not bind and metabolize human plasma LDL, which contain predominantly apo B. Since rat cells are known to have receptors for rat LDL it is possible that the primary structure of human apo B may have been altered to a great extent during evolution, whereas the structure of human apo E is not significantly different from that of rat apo E.

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REFERENCES

Angel, A., D'Costa, M. A. & Yuen, R. (1979) Can. J. Biochem. **57**, 578–587

Angel, A. & Fong, B. (1983) in Adipocyte and Obesity: Cellular and Molecular Mechanisms (Angel, A., Hollenberg, C. H. & Roncari, D. A. K., eds.), pp. 179–190, Raven Press, New York

Bilheimer, D., Eisenberg, S. & Levy, R. I. (1972) Biochim. Biophys. Acta **260**, 212–217

Bradley, W. A. & Gianturco, S. H. (1986) J. Lipid Res. **27**, 40–48

Bradley, W. A., Hwang, S. C., Karlin, J. B., Lin, A. H. Y., Prasad, S. C., Gotto, A. M., Jr. & Gianturco, S. H. (1984) J. Biol. Chem. **259**, 14728–14735

Brown, M. S., & Goldstein, J. L. (1974) Proc. Natl. Acad. Sci. U.S.A. **71**, 788–792

Chajek-Shaul, T., Friedman, G., Stein, O., Olivecrona, T. & Stein, Y. (1982) Biochim. Biophys. Acta **712**, 200–210

Desai, K. S., Steiner, G., Takeuchi, I. & Hollenberg, C. H. (1980) Biochim. Biophys. Acta **620**, 341–351

Drevon, C. A., Attie, A. D., Pangburn, S. H. & Steinberg, D. (1981) J. Lipid Res. **22**, 37–46

Farkas, J., Angel, A. & Avigan, M. I. (1973) J. Lipid Res. **14**, 344–356

Fong, B. S., Rodrigues, P. O. & Angel, A. (1984) J. Biol. Chem. **259**, 10168–10174

Goldstein, J. L. & Brown, M. S. (1983) The Metabolic Basis of Inherited Disease, 5th edn., pp. 672–712, McGraw-Hill, New York

Goldstein, J. L., Brunschede, G. Y. & Brown, M. S. (1975) J. Biol. Chem. **250**, 7854–7862

Goldstein, J. L., Basu, S. K., Brunschede, G. Y. & Brown, M. S. (1976) Cell **7**, 85–95

Goldstein, J. L., Brown, M. S. & Anderson, R. G. W. (1977) in International Cell Biology (Brinkley, B. R. & Porter, K. R., eds.), pp. 639–647, Rockefeller University Press, New York

Goldstein, J. L., Faust, J. H., Chorvat, R. J. & Brown, M. S. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 1877–1881

Gunther, S., Alexander, R. W., Atkinson, W. J. & Gimbrone, M. A., Jr. (1982) J. Cell Biol. **92**, 289–298

Innerarity, T. L., Pitas, R. E. & Mahley, R. W. (1979) J. Biol. Chem. **254**, 4186–4190

Innerarity, T. L., Pitas, R. E. & Mahley, R. W. (1980) J. Biol. Chem. **255**, 11163–11172

Krause, B. R. & Hartman, A. D. (1984) J. Lipid Res. **25**, 97–110

Nilsson-Ehle, P. & Schotz, M. C. (1976) J. Lipid Res. **17**, 536–541

Radding, C. M., & Steinberg, D. (1960) J. Clin. Invest. **39**, 1560–1569

Scow, R. O., Chernick, S. S. & Fleck, T. R. (1977) Biochim. Biophys. Acta **487**, 297–306

Stein, O., Freidman, G., Chajek-Shaul, T., Halperin, G., Olivecrona, T. & Stein, Y. (1983) Biochim. Biophys. Acta **750**, 306–316

Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354

Weisgraber, K. H., Mahley, R. W. & Assmann, G. (1977) Atherosclerosis **28**, 121–140

Yamamoto, M., Ranganathan, S. & Kottke, B. A. (1985) Biochem. Int. **11**, 573–581

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